A COMPARISON OF A 2.26% FLUORIDE VARNISH VERSUS A 1.23% APF FOAM USING POLARIZED LIGHT MICROSCOPY, CONFOCAL MICROSCOPY AND QUANTITATIVE LIGHT FLUORESCENCE

by

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INTRODUCTION

Secondary caries and the replacement of existing restorations account for 50 to 70 percent of operative dentistry today.[1-3] Restorative dentists have long recognized this fact, yet demineralization with subsequent formation of secondary caries at the restoration margin continues to be a problem. In the past few years, growing attention has focused on early detection of incipient lesions.[4-9] For most clinicians, detection of recurrent caries and incipient lesions is accomplished by clinical observation usually with the aid of a light-source, mirror, sharp explorer, and an air-water syringe. High quality radiographs are also helpful, but even with the improvements in radiographic techniques, traditional radiographic analysis does not allow for the detection of the early caries process. Gwinnett[10] found that for a lesion to be radiographically evident the lesion must have progressed to a minimum depth of 300-500 μ. Contributing to the problem is research suggesting that the use of an explorer in non-cavitated, but demineralized tooth structure may convert the early white spot lesion with an apparently sound surface layer into a cavity.[11] It is believed that by detecting the demineralized areas prior to frank cavitation, specific caries interception regimens can be initiated to allow the lesion to be arrested or even reversed. In an effort to detect these early lesions, promising research is currently being conducted in the use of quantitative light fluorescence, direct digital radiography, digital fiberoptic transillumination, and electroconductivity measurements.[6] New fluoride regimens and fluoride products are also being studied to intercept these early carious lesions.[12,13]

REVIEW OF LITERATURE

TOPICAL FLUORIDE

In an effort to reverse or arrest incipient carious lesions, many studies have examined the effects of fluoride on bacterial plaque and demineralized tooth structure. An understanding of the demineralization process leading to a lesion is necessary in order to understand how the lesion can first be detected and then arrested or prevented. Margolis and Moreno[14] developed a plaque model to explain the demineralization process and the role that fluoride plays in the process leading to caries that is still in use today. Plaque microorganisms produce organic acids when presented with fermentable carbohydrates. These organic acids diffuse through the acquired pellicle into the pores of the sound enamel surface. When the right combination of pH, acid concentration, and degree of saturation are present, dissolution of the enamel surface occurs. As the acid diffuses, less-soluble phases of dicalcium phosphate dihydrate and fluoridated hydroxyapatite precipitate from the enamel surface layer. An equilibrium situation is then established between the outer enamel surface layer and the enamel pores. The thermodynamic driving forces cause the acidic components to diffuse from the pores of the outer enamel surface into the inner enamel regions and results in the dissolution of underlying enamel. Dissolved enamel components then diffuse back into the surface layer and induce the precipitation of mineral phases in the surface enamel. Dissolved mineral ions (calcium and phosphate) will diffuse out of the enamel surface into the oral environment in an equilibrium situation. Thus the surface layer remains unaltered and intact due to the ions diffusing from the developing lesion deep in the inner enamel.

When this process continues without a balanced exchange of mineral ions, the lesion progresses until it eventually cavitates. The initial white spot lesion that is produced is caused by an optical effect created by the loss of inner enamel ions and becomes exaggerated when the tooth is dried. Margolis and Moreno concluded that sufficient concentrations of free fluoride ion promote the remineralization of enamel and the deposition of fluoride-containing mineral phases within resting plaque. Under acidic conditions, fluoride reduces the rate of enamel demineralization by fostering the precipitation of fluoridated hydroxyapatite phases within the surface layer of enamel.

Other studies have shown that concentrations of free fluoride ion are necessary for enamel remineralization. Wefel and Harless[15] argued that the effects of topical fluoride treatments on the caries process occurred when fluoride became permanently bound in the enamel. Feagin and coworkers [16] believed that fluoride deposition was optimal when sufficient time was allowed for the fluoride ion to exchange with the hydroxide ions in a "maturation process" in the enamel. Another study[17] found that the fluoride uptake into enamel surfaces was directly affected by the amount of time the fluoride was in contact with the enamel. In effect, the longer the fluoride remains in contact with the tooth, the greater the uptake of fluoride in the enamel and the greater the caries prevention. It became apparent that some fluoride agents seemed to be more effective at inhibiting the caries process. Differences seemed to exist in the effect of fluoride treatments and this appeared to be based on the mode of the fluoride delivery. Fluoride foams, varnishes, gels and rinses have been studied and compared in an effort to discover which vehicle provides the most effective caries reduction. Wefel and Harless[18] reasoned that the differences in the effect of some of these fluoride agents may be due to the release of

fluoride at a differential rate and this may be responsible for the caries preventive effects seen *in vivo.* They also believed that low concentrations of fluoride might ensure the presence of fluoride at a time when the white spot lesion is in the early stage of development and thus help to promote the remineralization of enamel.[19] Silverstone et al.[20] found a greater-than-four-fold increase in remineralization occurred when a low level of fluoride ions was added to a low-level calcifying solution. These experiments showed that small intact surface lesions in the human dental enamel were able to remineralize *in vitro.* If early caries detection is possible, then interceptive procedures may reverse the caries process.

FLUORIDE VARNISH

Fluoride varnish has been used in Europe as a topical fluoride agent for many years now. In the United States great interest has been focused on the potential fluoride varnish has for reducing caries. The first sodium fluoride varnish contained 5 percent sodium fluoride by weight and 2.26 percent fluoride ion by weight in a natural colophony base.[21] It is this base that provides the yellow color and the adhesive properties seen in some fluoride varnishes and allows the sodium fluoride to remain in contact with the tooth for longer periods of time. The manufacturer recommends that the varnish remain in contact with the tooth for at least two hours before toothbrush removal. Once the varnish sets, the fluoride released from a varnish may be released at a slower and more continuous rate than other topical fluoride agents. This may inhibit the caries rate more significantly than other fluoride treatment products and allow the maturation process, which Feagin et al.[16] discussed, to occur. One study examined the depth of fluoride

penetration into the enamel surface when fluoride varnish was applied. These researchers[22] found that fluoride varnish produces a permanent deposit of fluoride not only at the surface but also at depths of 50 μ or more. They believed this to be due to a longer effective time for reaction with the hydroxyapatite in the enamel, which allowed the fluoridated surface and subsurface enamel to serve as a reservoir for fluoride release. If fluoride varnish remains in contact with the tooth for longer periods of time than traditional fluoride treatment regimens, more fluoride may be permanently bound and a decrease in the amount of demineralization might occur. This may be particularly true when the varnish is applied to a cavity preparation prior to condensation of an amalgam, because the amalgam restoration may shield the varnish from rapid dissolution. Fluoride varnish is a highly concentrated fluoride agent with 1 ml equal to 23 mg fluoride. This raises the question of toxicity, particularly in the pediatric population. However, researchers have found that the amount needed to cover the tooth surfaces in children are far less than 1 ml.[23] This study found that the fluoride dose was approximately 3.0 mg for young children and 5.0 mg for older children. Cousins and Mazze[24] found that the nephrotoxic threshold of fluoride is 850 ng/ml of fluoride in plasma. At these doses, the highest plasma-fluoride concentration varied between 60 ng/ml and 120 ng/ml of fluoride. These plasma levels were far below those considered toxic. These researchers concluded that when the recommended dosages of fluoride varnishes are followed, no toxic side effects should occur.[23] Although uncommon, fluoride varnish has been found to cause allergic hypersensitivity reactions in a very small percentage of the population.[24]

Many studies have been designed to compare fluoride varnish with other accepted fluoride treatment regimens. The application of fluoride varnish to the permanent

dentition has shown caries reduction rates ranging from 32.5 percent to 61.7 percent versus untreated controls.[26-29] Significant reductions in the caries rate were also demonstrated when fluoride varnish was applied at least semi-annually versus a single yearly application.[27,31] When fluoride varnish has been compared in half-mouth studies, caries reductions of 24 percent to 30 percent have been shown when the varnish was applied semi-annually.[32,33] These studies seem to demonstrate that semi-annual fluoride varnish applications can significantly reduce the rate of caries in the permanent dentition.

The use of fluoride varnish has also been examined in the primary dentition. Studies have examined fluoride varnish when it was applied annually, semi-annually and in half-mouth studies. The results demonstrate reductions in the caries rate ranging from 11.6 percent to 36.6 percent.[34-38] Murray and coworkers[38] performed a study to examine the caries rates in primary teeth and newly erupted permanent molars. The results showed a 7.4 percent decrease in diseased, missing, and filled surfaces when subjects received the fluoride varnish in semi-annual applications versus the untreated side of the mouth. In newly erupted molars, a 36.6 percent decrease in the caries rate was found in the fluoride varnish treated molar teeth versus the untreated control side of the mouth. These studies seem to suggest that fluoride varnish treatments are somewhat less effective in the primary versus the permanent dentition, although a beneficial reduction in the caries rate can be expected when fluoride varnish is applied to the primary teeth.

Another study was performed in which Tewari and coworkers[39] compared the effectiveness of fluoride varnish against 2 percent sodium fluoride, and 1.23-percent APF solution used as semi-annual fluoride applications. The fluoride varnish showed a 65 percent greater reduction in the caries rate compared with the 2.0 percent sodium fluoride

and a 61 percent greater reduction in the caries rate compared with the 1.23-percent APF solution. This study indicates that fluoride varnish may be more effective than 2.0 percent sodium fluoride solutions and 1.23-percent APF solution. When compared directly against 1.23-percent APF gel, two studies have shown the fluoride varnish to provide a significant decrease in the caries rate ranging between 11.7 and 25.5 percent.[40,41] The previous data have shown the efficacy of fluoride varnish versus 1.23-percent topical APF foam.

Tveit[42] performed an important *in vitro* study that has implications for inhibiting secondary caries. Tveit placed Fluor Protector (Vivacare/Vivadent, Schaan, Liechtenstein), a 0.1-percent fluoride as a difluorosilane in a polyurethane varnish base, adjacent to 12 amalgam restorations. An electron probe showed that there was significant fluoride uptake into the enamel tooth structure (25,000 ppm) and moderate uptake into the dentin walls (7,000 ppm) compared with the untreated half of the restoration that acted as a control. By placing fluoride varnish at the tooth-restoration interface, a positive effect on the inhibition of white spot lesions and subsequent secondary decay adjacent to amalgam restorations may be achieved.

Donly and colleagues[43] examined the effect of fluoride on the formation of wall lesions in an *in vitro* study using 1.23-percent APF foam. This study showed that an application of fluoride foam for 4 minutes versus 1 minute in the cavity preparations prior to amalgam condensation resulted in significantly less demineralization at the restoration margin than in the untreated control. No significant difference was found between the fluoride placed for 1 minute versus the fluoride placed for 4 minutes. *In vitro* acid challenges have been used to produce demineralization and wall lesions adjacent to

restoration margins in many studies with the data being analyzed with polarized light or confocal microscopy.[20,43-49]

Gonzalez and colleagues[50] examined demineralization at the restoration margin when a fluoride varnish is placed into a cavity preparation prior to amalgam condensation. These researchers concluded that fluoride varnish delayed the formation of secondary caries around amalgam restorations. Marchori et al.[51] performed a study that demonstrated that fluoride varnish exhibited significant microleakage when the cavity margins remained in enamel. Using Tveit's data, we hypothesize that fluoride applied to the cavity preparation will be taken up by the cavity wall and cause a decrease in demineralization and subsequent decrease in secondary caries formation at the restoration margin. Donly and his colleagues[43] have compared the effect of topical fluoride before and after amalgam placement on the inhibition of caries. Using their study as a framework, this thesis was designed to compare placement regimens of fluoride varnish and 1.23-percent APF foam prior to and following amalgam restoration placement and then determine the differences in demineralization adjacent to the amalgam restoration.

Once these incipient lesions were created, they were evaluated using confocal microscopy, polarized light microscopy and quantitative light fluorescence.

CONFOCAL MICROSCOPY

Researchers have examined carious lesions and the effectiveness of interceptive treatments with the use of confocal microscopy.[52,53] Specimens can be viewed with confocal microscopy by sectioning the tooth and then hydrating the tooth with a fluorescent medium (ex: Rhodamine B). Gonzalez-Cabezas et al.[54] found that the use of

confocal laser scanning microscopy is an effective technique for measuring *in vitro* mineral changes in dental tissues. Confocal microscopy operates on the principle that demineralized tooth structure contains larger pores than sound tooth structure. These pores can be penetrated with a dye that will differentially fluoresce, depending on the amount of dye present. Greater demineralization causes the tooth structure to become more porous and allows more dye penetration. Pore volume is the volume of fluid that has penetrated these pores of the tooth. An increase in pore volume may indicate increased demineralization. A decrease in pore volume may indicate less demineralization or, in some cases, a remineralization process.

Fluorescence of the Rhodamine B dye occurs when the dye is illuminated with an ion argon laser using a 488-nm excitation wavelength. Benn and Watson[55] used Rhodamine blue as the dye to measure the depth of natural carious lesions and found a correlation of lesion size of confocal images and backscattered electron images. Attenuating the output from the laser using a range of neutral density filters to achieve the 488-nm excitation wavelength controls the amount of light reaching the specimen. Rhodamine blue is a fluorescent dye, which, when struck with this wavelength of light, will act as a signal from the specimen to a photomultiplier tube positioned just behind a pinhole. The photomultiplier tube detects only light focused at the pinhole; light from above and below the plane of interest in the specimen is prevented by the pinhole from striking the photomultiplier tube.[56] The image is formed by recording light primarily from a small focal volume, largely ignoring points to the side or above or below. That volume, described as a point spread function, is the product of two similar functions that are generated by the objective lens. Because of that multiplication, the recorded light is

greater than even the integrated total of the light from all other points in a thick sample.[57] The output from the photomultiplier tube is built up in a digital framestore in a microcomputer; it is displayed as an image on a video monitor screen or stored as a digital file. Images are collected at approximately one frame per second and signal averaging is usually necessary to eliminate much of the background noise in the images.[56]

POLARIZED LIGHT MICROSCOPY

Polarized light microscopy is another tool used by researchers to examine the depth of lesion progression after the tooth has been sectioned and imbibed in an aqueous medium. In contrast to confocal microscopy, polarized light microscopy is able to further classify white spot lesions into four distinct zones, and it is useful for describing the early caries lesion and the alterations in structure upon further demineralization or remineralization. Research has demonstrated that small changes in the optical properties of the tissue could be determined with polarized light before qualitative microradiographic changes were observed.[58] Polarized light microscopy is based on the principle that light travels at different velocities through materials of different molecular structure. If light is transmitted with equal velocity in all directions in a crystal, the crystal is said to be isotropic. However, if light is transmitted at different velocities in certain directions, the crystal is termed anisotropic. Anisotropic crystals are further said to be uniaxial, and biaxial, depending upon the number of axes present. Hydroxyapatite is uniaxial, having one optic axis coincident with its crystal axis. Hydroxyapatite is also termed birefringent. Birefringence is a descriptive term given to materials that are able to cause light to travel at two different velocities and thereby demonstrate two principal

refractive indices.[59]

Polarized light microscopy is used to measure birefringence indirectly. A polarizing light microscope is a light microscope, to which a polarizer and analyzer have been added, each of which is made of prisms of calcite or a sheet of Polaroid. Each of these will transmit light oscillating in one plane.[60] If a uniaxial, birefringent object is placed on the stage of a polarizing light microscope with its optic axis parallel to the direction of plane-polarized light propagation, the object will behave as an isotropic crystal. If the object is arranged with its optic axis in a plane perpendicular to the direction of propagation, the light is split into two beams. These two beams are known as the ordinary and extraordinary rays. The two rays vibrate in mutually perpendicular planes along which the refractive index is termed n_o for the ordinary ray, and n_e for the extraordinary ray. The optical system is arranged to cause interference of these two rays and the end result is seen as a series of interference colors.[59]

Enamel consists chiefly of inorganic hydroxyapatite and a small amount of organic material. The sign of birefringence of the inorganic component is negative with respect to the prism length. Inorganic enamel prisms have negative birefringence. The organic portion of natural enamel is positively birefringent, but is so minimal that it is disregarded.[59] With non-cubic crystals a sign of birefringence is given to the structure, determined by the path taken by the slower (+) and faster (-) light rays in relation to the morphology of the crystal.[60]

Intrinsic birefringence is the difference between the refractive indices of the extraordinary ray and the ordinary ray, or (n_e-n_o).[61] Factors that influence intrinsic birefringence include the volume of crystalline mineral, the crystal lattice arrangement

and the inclination with respect to the light beam, and the birefringence of crystallites, so that:

$$(n_e\text{-}n_o)\ i = \$c\ (n_e\text{-}n_o)\ hap,$$

where $(n_e\text{-}n_o)$ i = intrinsic birefringence; \$ = pore volume occupied by crystallites; c = crystallite orientation factor and $(n_e\text{-}n_o)$, and hap = birefringence of crystallites.[58]

During both formation and carious breakdown of sound enamel, a considerable number of spaces exist between the crystals and the prisms. These spaces located between the crystals are small compared with the wavelength of light and can give rise to an additional type of birefringence, known as form birefringence. The amount of form birefringence produced depends upon the percentage of spaces present in the tissue and the refractive index of the medium contained within the spaces. If the medium filling the spaces has a refractive index equal to that of the enamel crystals, then no form birefringence will be produced and the tissue will exhibit only the intrinsic birefringence of enamel.[59] When a medium of refractive index different from that of the enamel rods is used, the form birefringence produced will be positive in sign with respect to the prism length, because the crystals lie with their optic axes approximately parallel to the prism length.

Total birefringence is composed primarily of intrinsic birefringence and form birefringence. The observed birefringence of enamel will be the sum of; (1) the negative intrinsic birefringence of inorganic material; (2) the positive intrinsic birefringence of the organic material; (3) the positive form birefringence of the spaces in relation to the mineral and possibly the organic material, and (4) the positive form birefringence of the organic material in relation to the mineral. As stated before, for practical purposes, the

observed birefringence is made up almost entirely of the intrinsic birefringence of the inorganic mineral and the form birefringence of the spaces in relation to the inorganic mineral. This is because the organic mineral is small and can be disregarded.[59] When the spaces, or pores, are filled with a medium having the same refractive index as enamel, the form birefringence will be eliminated. Therefore, when enamel is viewed in longitudinal ground sections with a polarizing light microscope, the image formed depends upon the refractive index of the mounting medium and its degree of penetration into the tissue. The greater the difference between the refractive index of the mounting medium and the enamel, the more positive form birefringence will be produced.[60]

Longitudinal sections of enamel are used in order to align crystallites of apatite along the length of the prism. Four zones of demineralization can be seen when viewing longitudinal enamel lesions subjected to artificial caries challenge under polarized light. In polarized light, the percentage of pore volume present in each zone differentiates the enamel lesion into the four zones. The most advanced translucent zone has 1 percent of relatively large spaces. The dark zone, just superficial to the translucent zone, has 2 to 4 percent of the large pore space in a background of micropores and exhibits positive birefringence when the section is examined in quinoline. The body of the lesion has from 5 to 25 percent large pore volume that is accessible to all imbibition media. The unchanged superficial zone has 1 to 5 percent pore volume and shows negative birefringence with water and polarized light microscopy.[61] This zone acts as a well-mineralized barrier where remineralization of the lesion occurs[20] (FIGURE 1). The superficial, dark, and translucent zones act as sieves, which selectively exclude the imbibition medium with molecular sizes greater than pore sizes present in these zones.[61]

Different imbibition media with different refractive indices are used to observe sections of demineralized enamel. Based on the refractive indices of the imbibition media, the percentage of pore volume in a given enamel lesion can be evaluated. Imbibition medium include aqueous solutions of water (refractive index = 1.33) and a series of potassium mercuric iodide dilutions (Thoulet's solution) with refractive indices of 1.41, 1.47, and 1.62.[61] Air corresponds with 1.0-percent pore volume, water with 5.0-percent pore volume, Thoulet's 1.41 with 10-percent pore volume, and Thoulet's 1.47 with 25-percent pore volume. Only air (refractive index 1.0), water, and methanol can be used to imbibe sound enamel. Silverstone et al.[20] has also used water and quinoline (refractive index 1.62) to view the four zones of demineralization. They observed the superficial zone and body portions of the lesion with water and the dark and translucent zones were observed with quinoline. As demineralization increases, pore volume increases, and the selection of an imbibition medium is based on the amount of pore volume present in a lesion. The greater the demineralization pore volume, the higher the refractive index of the imbibition medium that is used to evaluate the lesion. By systematically substituting the media listed above, one can determine which area of the lesion corresponds to a specific range of pore volume. Because air and water allow for evaluation of 1.0- and 5.0-percent pore volumes, respectively, and the innermost advanced front of the lesion has a pore volume of 1 percent, the positively birefringent lesion shown with air and water will appear the largest. Water, Thoulet's 1.41, and Thoulet's 1.47 reveal pore volumes of 5 percent, 10 percent, and 25 percent, respectively. Consequently, the positively birefringent lesion appears progressively smaller as the refractive indices of the media increase and the pore volumes they indicate become

larger.

Internal lesion pore volume can be identified utilizing the information found by imbibing the lesions with media of increasing refractive indices. A series of photomicrographs of the lesion imbibed in the various media are superimposed to render a contour map revealing the location and extent of the lesion's mineral loss. Contour maps provide a qualitative assessment of internal lesion pore volume, and quantitative information can be easily obtained by measuring the positively birefringent areas of the lesion in the various imbibition media. The polarized light photomicrograph is projected onto the tablet of an image analysis system and carefully quantitated. It is relatively simple to quantify lesion parameters and evaluate the data with statistical software. Although the polarized light microscope does not directly provide quantitative data by this method, measurements of the images are quantifiable indirectly.[61] It should be noted that the quantified measurements are not indicative of quantitative changes in pore volume.

Confocal microscopy and polarized light microscopy are used as the gold standards in caries research for evaluating incipient carious lesions. Quantitative light fluorescence is a new technique that may allow incipient caries to be clinically detected intraorally.

QUANTITATIVE LIGHT FLUORESCENCE

Different methods have been examined for detection of early carious lesions. One of the most promising early caries detection systems is the Quantitative Light Fluorescence system or QLF (Inspektor Research Systems, the Netherlands). The

interest in QLF may be due to the many advantages it has over traditional techniques for detecting and monitoring incipient lesions over time. These advantages include that: no dye or aqueous medium is needed to imbibe the teeth in order to examine the lesion; the teeth do not need to be extracted and sectioned in order to examine lesion progression; QLF can be done *in vivo.* Until recently, researchers traditionally had only two ways to evaluate incipient lesions and secondary caries. *In vitro* studies artificially created lesions and then sectioned the teeth and quantified the lesions using various techniques including confocal microscopy and polarized light microscopy. *In vivo* studies perform treatment intraorally and then collect the teeth upon exfoliation in order to evaluate the treatment effect. These studies are limited in their diagnostic ability, because the teeth must be removed from the oral environment prior to examination.

QLF was designed to measure the loss of fluorescence of a carious lesion by illuminating the tooth with a beam of 290 to 450 nm of light. This light may be absorbed by chromophores in the enamel causing visible fluorescence. Carious lesions have lower numbers of chromophores, and there is less fluorescence. This causes the carious lesion to appear darker than sound enamel.[62] This technology has been developed into an intra-oral light fluorescence system that uses QLF to assess the baseline mineral content and longitudinal change in mineral content in early enamel lesions *in vivo*.[63] The system currently uses an arc lamp, filtered to a small band (370 +/- 80 nm), and a camera control unit, which is connected via a liquid light guide and electrical cable to a hand-held intra-oral camera (FIGURE 2). The image of the area can then be saved and analyzed. The system integration and software are similar to the laser-based version of QLF.[64] It has been demonstrated that QLF could detect *in vitro* mineral changes of enamel around

amalgam restorations.[65] Recently, pilot studies have examined the ability of QLF to detect white spot lesions *in vivo*. A six-month pilot study examined the effect of fluoride varnish treatment on remineralization of white spot lesions in caries-active children using QLF. It was concluded that repeated fluoride varnish application increased the remineralization of white spot lesions and that QLF was suitable for evaluation of preventive measures in short-term studies.[66] Zandona et al.[67] examined 150 children nine to 12 years of age living in a non-fluoridated community using QLF. They concluded that QLF diagnosed more lesions than clinical examination, was reproducible, and was able to monitor lesions over time. While studies have shown that QLF can detect incipient smooth surface caries and secondary caries in enamel, no study has yet examined the ability of QLF to detect secondary caries in dentin.

PURPOSE

The study was designed to accomplish three purposes. First, and most important, this study was designed to determine if QLF could detect incipient secondary lesions in both enamel and dentin that could be verified with two traditional techniques: confocal microscopy and polarized light microscopy. Second, we wanted to determine the differences in the amounts of demineralization at the restoration margins between teeth treated with a fluoride varnish and 1.23-percent APF foam. Third, we chose to determine whether placing fluoride varnish and 1.23-percent APF prior to amalgam condensation would have a beneficial impact on the demineralization processes.

HYPOTHESES

The present study was designed to test three hypotheses:

1) Fluoride varnish would be more effective than 1.23-percent APF foam at inhibiting demineralization at the amalgam restoration-tooth interface both pre and post-condensation.

2) Application of a fluoride varnish prior to the condensation of amalgam would result in less demineralization at the restoration-tooth interface than application of fluoride varnish following placement of the amalgam restoration.

3) Quantitative Light Fluorescence (QLF) would be able to detect early incipient secondary caries as accurately as confocal microscopy and polarized light microscopy.

MATERIALS AND METHODS

TOOTH SELECTION AND PREPARATION

Seventy-five extracted, caries-free human mandibular molars were selected for the study. All teeth were disinfected in a buffered 10-percent formalin solution for a minimum of three weeks. All calculus, bone, and soft tissues were removed and the teeth cleaned with a prophy cup, non-fluoridated pumice, and deionized water slurry. Cusp tips of all teeth were flattened for ease of sectioning and painted with a clear, acid resistant varnish covering all surfaces of the teeth except 1 mm directly adjacent to the margin of the alloy restoration. Standard Class V restorations were placed on the mesial surface of each tooth at the cementoenamel junction using a #330 carbide bur in a high-speed handpiece with water coolant. Preparations extended 6 mm buccolingually, 4 mm occlusogingivally and 1.5 mm axially in depth. Each cavity preparation had the occlusal margin in enamel and the gingival margin in cementum. Amalgam (Tytin-Kerr, Romulus, MI, USA) restorations were then placed following standard amalgam placement techniques.[68] Teeth were randomly assigned a number and placed into five treatment groups.

TREATMENT REGIMENS

Group A (Varnish, Precondensation):

Fifteen teeth had a fluoride varnish (Duraflor, 5 percent NaF, Pharmascience Inc.,

Montreal, Canada) placed into the cavity preparation. The varnish was allowed to air dry for five minutes and air was gently applied to thin the varnish prior to condensation of amalgam.

Group B (Foam, Precondensation):

Fifteen teeth had a 1.23-percent APF (acidulated phosphate fluoride) foam topical fluoride (Oral B Laboratories, Belmont, CA, USA) placed for 1 minute into the cavity preparation. The preparation was gently dried with air prior to the condensation of amalgam, purposely not rinsing the topical fluoride from the preparation.

Group C (Varnish, Post-condensation):

Following Class V alloy cavity preparation and condensation of amalgam, fifteen teeth had a fluoride varnish painted over the restoration margins. The varnish was allowed to air dry for five minutes and then air was gently applied.

Group D (Foam, Post-condensation):

Following Class V alloy cavity preparation and condensation of amalgam, fifteen teeth had a 1.23-percent APF foam topical fluoride applied to the restoration margins for 1 minute. The teeth were then gently dried with air.

Group E (Negative Control):

Fifteen teeth with Class V amalgam restorations acted as a negative control group with no fluoride varnish or APF foam applied.

After cavity preparation, fluoride application (where applicable), and amalgam placement, all five groups had a hole drilled into the root of the tooth. The tooth was suspended by dental floss into an artificial saliva solution and stored at room temperature, neutral pH, and constant circulation. The artificial saliva solution contained $NaHCO_3$ (20 mM), $NaH_2PO_4 \cdot H_20$ (3mM), and $CaCl_2 \cdot 2H_20$ in deionized water to produce two liters of solution. After 24 hours in the artificial saliva solution, all restorations, regardless of treatment, were brushed with a soft toothbrush and deionized water for two minutes to remove any residue.

CARIES CHALLENGE

All five treatment groups were placed into an artificial caries challenge solution for a duration of five days. The artificial caries challenge contained 2.2 mM $CaCl_2 \cdot 2H_20$, 2.2 mM KH_2PO_4, and 50 mM concentrated acetic acid in deionized water to produce 1.0 l of solution. This caused demineralization to occur at the tooth-restoration interface and allowed the formation of secondary caries in the samples (FIGURE 3). Following wall lesion formation, all specimens were rinsed and stored in deionized water to arrest further demineralization and remineralization until final analysis was completed.

QLF IMAGES AND ANALYSIS

The acid resistant nail varnish was carefully removed prior to QLF analysis to expose sound tooth structure. All restorations in the five groups were analyzed using quantitative light fluorescence (QLF). Images of all specimens' enamel fluorescence were taken using the QLF system (Inspekter Research Systems, Amsterdam, The Netherlands). Specimens were exposed to a 13 mW/cm^2 of violet-blue light (wavelength: 290 to 450 nm) via a liquid light guide in the camera handpiece. One image of enamel and one image of dentin were captured through a 510-nm band-pass filter, using a miniature CCD camera located inside the handpiece (FIGURE 4). The images were stored in the QLF 1.96w program.

In order to calculate fluorescence loss, a computer-generated rectangle (inner patch) was placed in the area of analysis. The size of the rectangle was kept constant for all specimens. The fluorescence values of the pixels within the rectangle (demineralized window) were compared with the fluorescence values of the pixels surrounding the rectangle (sound surrounding area, FIGURE 5). Lesion threshold was set at 95 percent, indicating that fluorescence values at less than 95 percent of their sound reference fluorescence values were considered demineralized. This difference was recorded as percent average change in fluorescence, maximum percent change in fluorescence, average fluorescence change per area in pixels, and as ^Q = average fluorescence change per area in mm^2.

PREPARATION AND DATA COLLECTION

Each tooth was mounted on a polyacrylic rod using methyl methacrylate resin and

dipped in casting resin prior to sectioning. The samples were sectioned using a Silverstone-Taylor hard tissue microtome (Scientific Fabrications Laboratory, Lafayette, CO.). The restorations were sectioned to a thickness of 90 to 120 μ.

Using polarized light microscopy, each specimen was imbibed with water, and the demineralized wall lesions adjacent to the amalgam restoration was photographed at X10 magnification and projected to a computer program for quantitization. Water was used as the imbibition medium in this study, because it allowed adequate visualization of the lesion area. Lesions seen in a water imbibition medium represent tooth structure that has greater than 5.0-percent pore volume, which correlates well with the lesion threshold of 95 percent that was used with the QLF analysis. Measurements at 50 μ and 100 μ were made perpendicular to the restoration and the image was saved into a computer program. Measurements were made at the enamel (FIGURE 6) and at the dentin margins (FIGURE 7). Data of the amounts of demineralization were obtained, and comparisons of the differences between the five groups were made.

Following polarized light microscopy evaluation, each section was stained overnight with a freshly prepared 0.1 mM Rhodamine B solution (Aldrich Chemical Co., Milwaukee, WI), without further rinsing. The stained surface was allowed to air dry for 10 minutes before being analyzed with the confocal laser scanning microscope (Odyssey, Noran Instruments, Inc., Middleton, WI). For each specimen, a measurement 100 μ in length was made in dentin perpendicular to the restoration. The enamel specimens were not measured due to the amount of demineralization and loss of tooth structure that was seen with polarized light microscopy. After being brought into focus (using an X10 Nikon objective, N.A. 0.25), the specimens were illuminated with an ion argon laser

using a 488 nm excitation wavelength. Confocal slits were set at 15 μ with a 515 nm long pass barrier filter, and the argon laser intensity was set at 100 percent. For collection of the images, samples were frame-averaged using 128 frames per image. Areas were scanned planoparallel to the transversal cut surface of the specimen and perpendicular to the natural surface of the tooth (FIGURE 8). Images of the area were computer saved and analyzed for area and total lesion fluorescence using Image 1 (version 4.14.C) software (Universal Images Corp., West Chester, PA).

STATISTICAL METHODS

The five groups were compared using one-way analysis of variance (ANOVA). Pairwise comparisons were made between the groups using Tukey's method to adjust for multiple comparisons. Contrasts were used to combine groups and make overall comparisons between varnish and foam and between pre-placement and post-placement application. The QLF measurements were compared for a difference from zero using a one-sample t-test to determine if the QLF measurements found a significant change.

RESULTS

QUANTITATIVE LIGHT FLUORESCENCE FOR DENTIN SPECIMENS

The control group had significantly higher lesion area: ($p < 0.05$), both expressed in pixels ($p = 0.0353$) (TABLE I) and in square millimeters (mm^2)($p = 0.0136$) (TABLES II-III) than the varnish pre-condensation group. The control had significantly higher lesion area: (in mm^2) than the APF foam post-condensation group ($p = 0.0488$). The control had significantly (higher) maximum change in fluorescence radiance ($p = 0.0255$) (TABLE IV), average change in fluorescence radiance ($p = 0.0101$) (TABLE V), and ^Q ($p = 0.0093$) (TABLE VI) than the varnish pre-condensation group. No other significant differences were found between the groups.

QUANTITATIVE LIGHT FLUORESCENCE FOR ENAMEL SPECIMENS

The groups were not significantly different for any of the QLF parameters: $p = 0.47$ for lesion area; pixels, $p = 0.50$ for lesion area: mm^2; $p = 0.86$ for maximum change in fluorescence radiance, $p = 0.86$ for average change in fluorescence radiance, and $p = 0.85$ for ^Q (TABLES I-VI).

POLARIZED LIGHT MICROSCOPY FOR DENTIN SPECIMENS

The varnish pre-condensation group had significantly higher demineralization at 50 μ than the APF foam pre-condensation group ($p = 0.0067$); no other significant differences were found between the groups using polarized light microscopy at 50 μ. The varnish pre-condensation group had significantly higher demineralization at 100 μ than

the control ($p = 0.0262$), the varnish post-condensation ($p = 0.0005$), the APF foam pre-condensation ($p = 0.0003$), and the APF foam post-condensation group ($p = 0.0352$) (TABLES VII-VIII).

POLARIZED LIGHT MICROSCOPY FOR ENAMEL SPECIMENS

There were no significant differences between groups using polarized light microscopy evaluation 50 μ from the restoration ($p = 0.82$) or using polarized light microscopy evaluation 100 μ from the restoration ($p = 0.82$) (TABLE VII).

CONFOCAL MICROSCOPY: DENTIN SPECIMENS

The control group had significantly higher total fluorescence values ($p = 0.03$), but not area values ($p = 0.06$), than the APF foam pre-condensation group. No other significant differences were found between the groups (TABLE IX-X).

CONFOCAL MICROSCOPY: ENAMEL SPECIMENS

Due to the amount of demineralization and loss of tooth structure seen with polarized light microscopy, a confocal microscopic analysis was not completed for enamel.

FIGURES AND TABLES

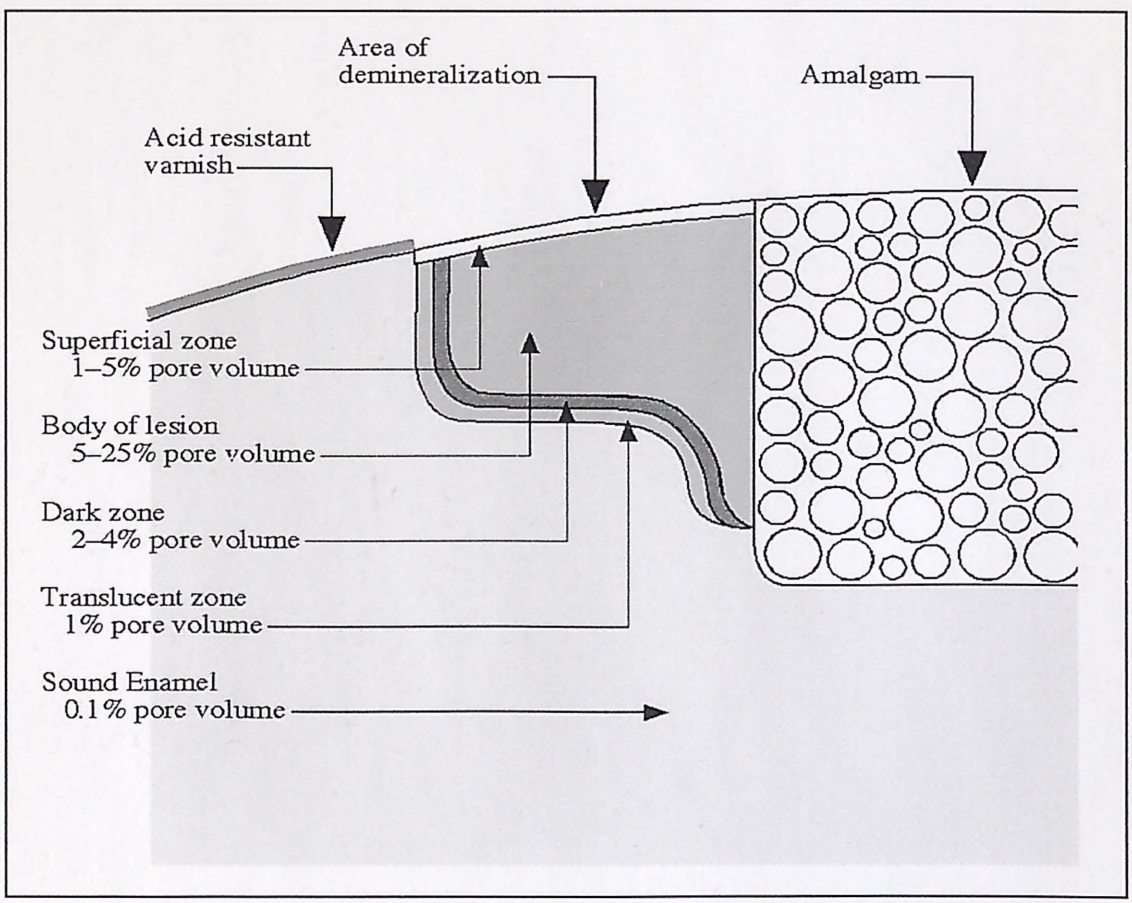


FIGURE 1. Histologic zones of an incipient lesion.

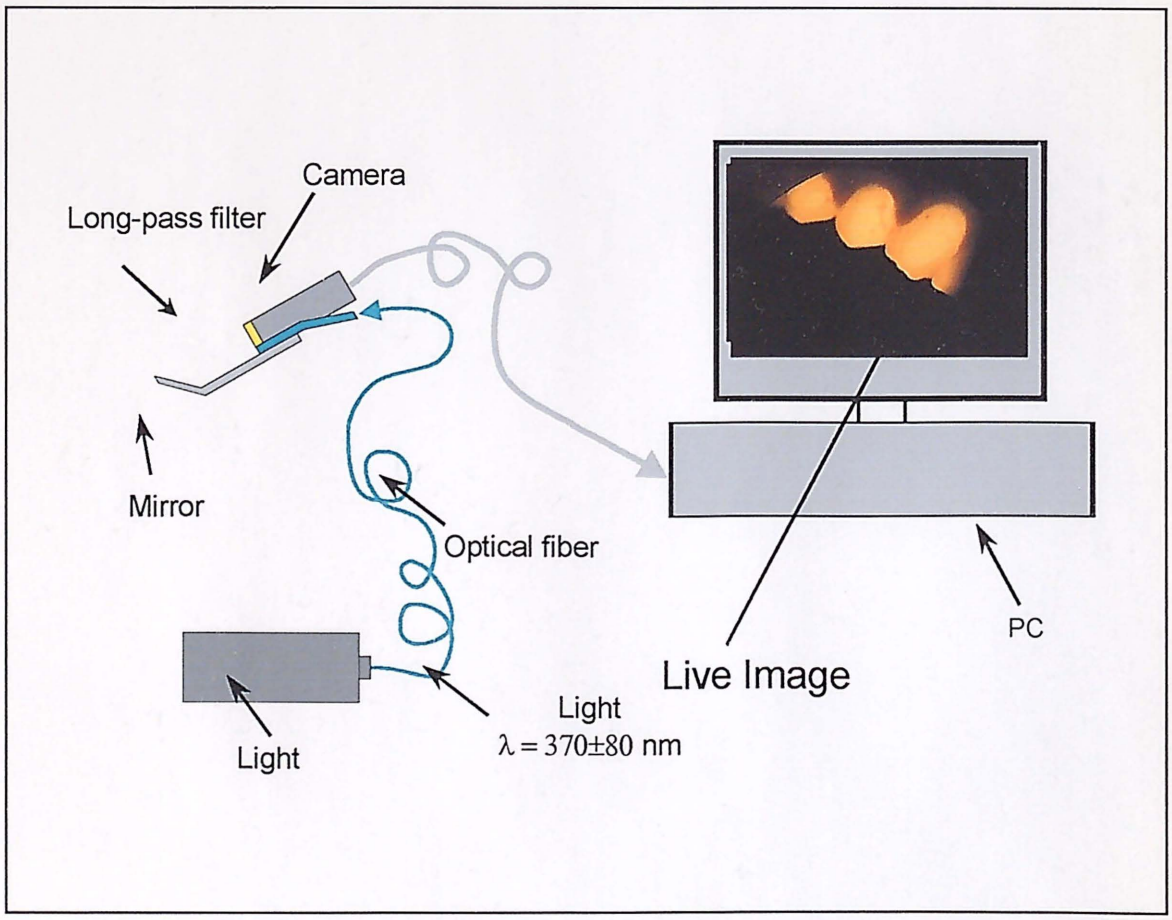


FIGURE 2. Diagram of QLF components.

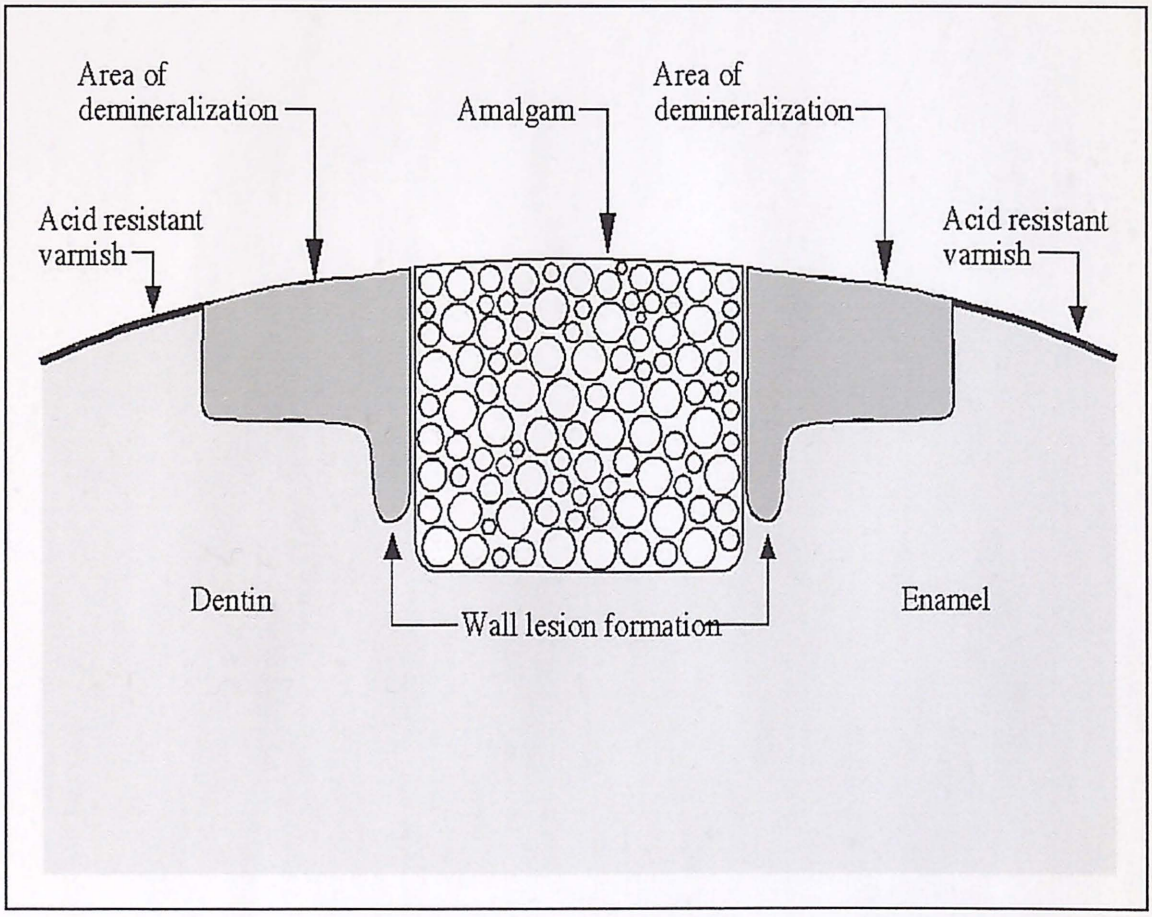


FIGURE 3. Secondary caries formation following artificial caries challenge.

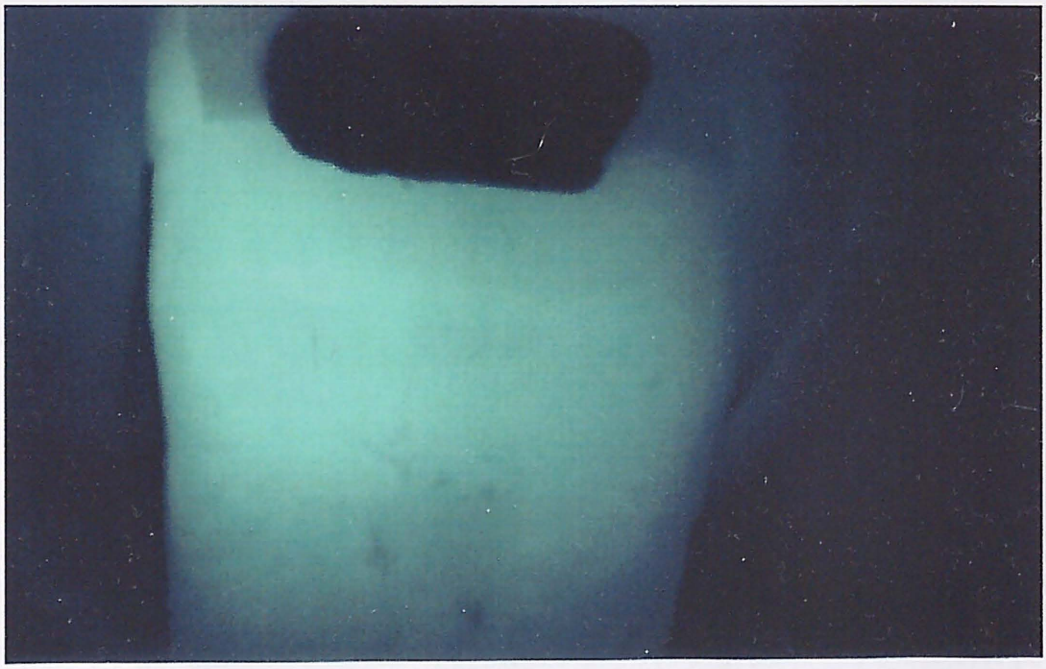


FIGURE 4. QLF: detection of enamel and dentin fluorescence change resulting from demineralization.

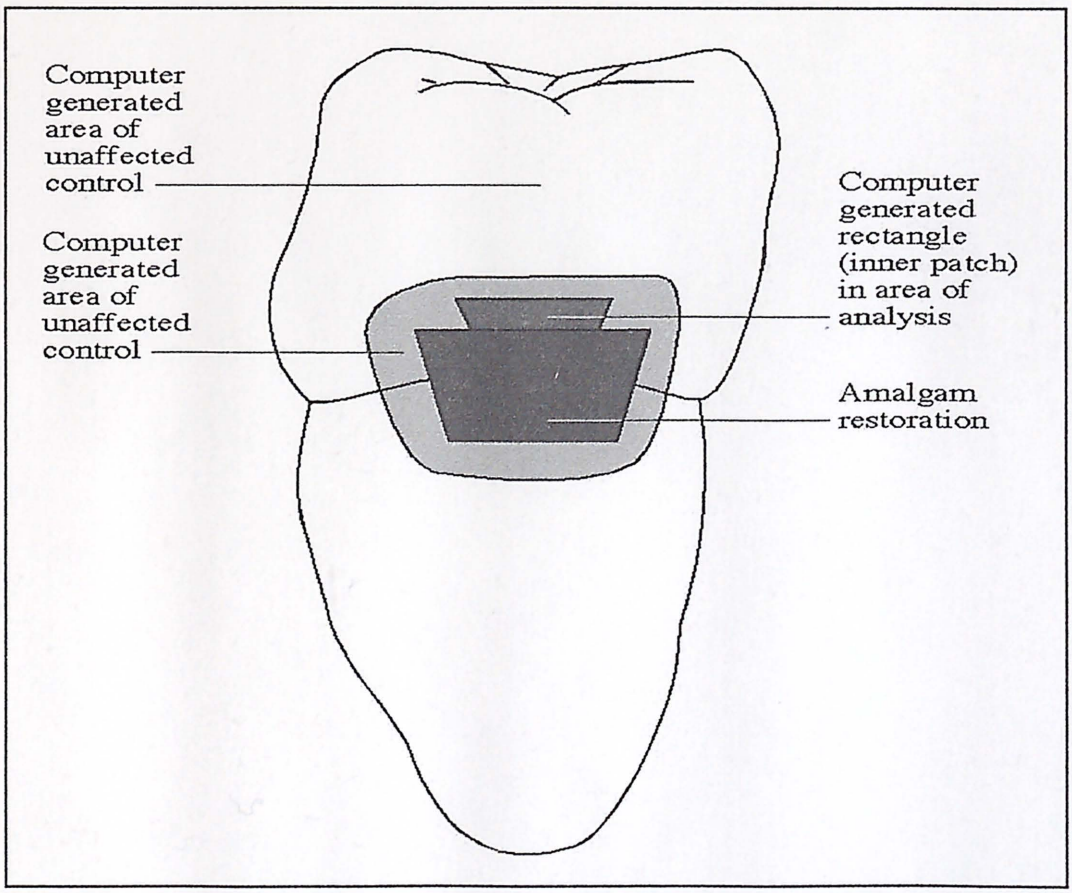


FIGURE 5. QLF: computer analysis.

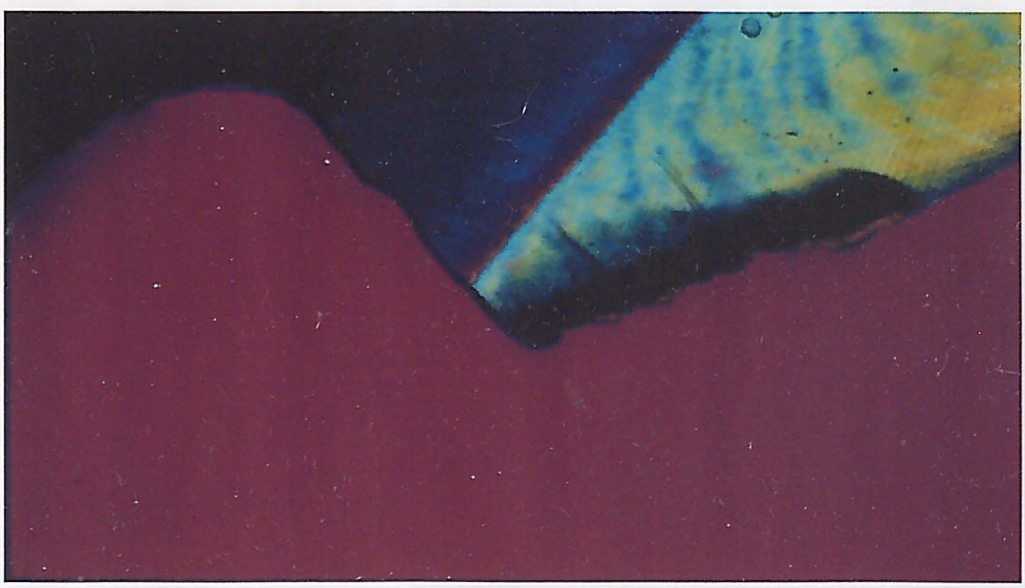


FIGURE 6. Polarized light microscopy: representative image of enamel.

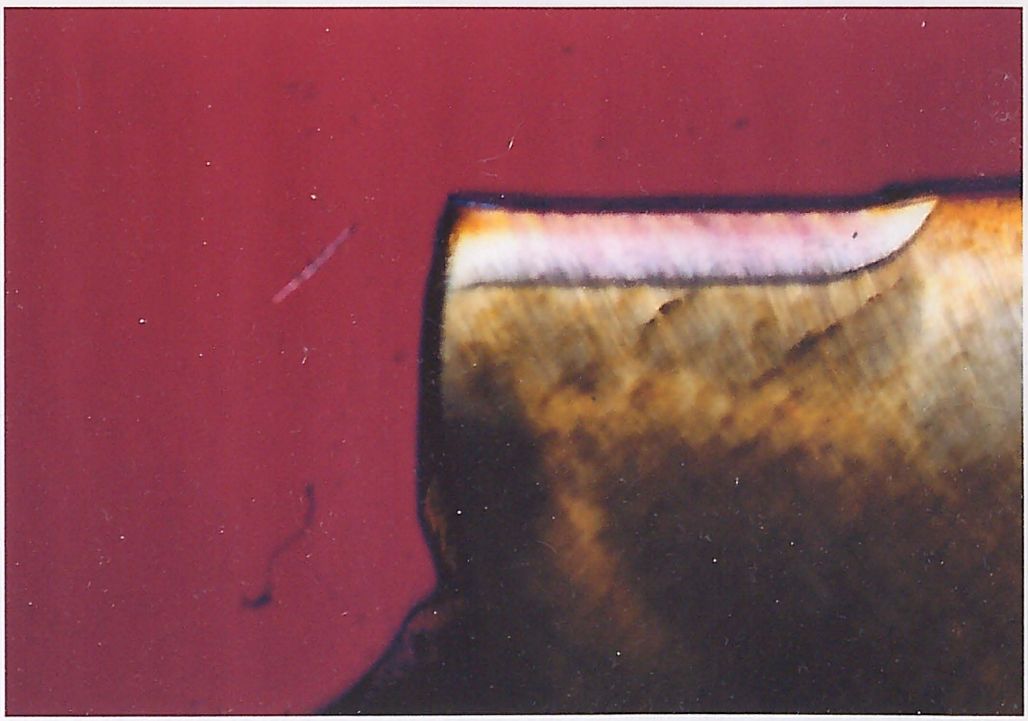


FIGURE 7. Polarized light microscopy: representative image of dentin.

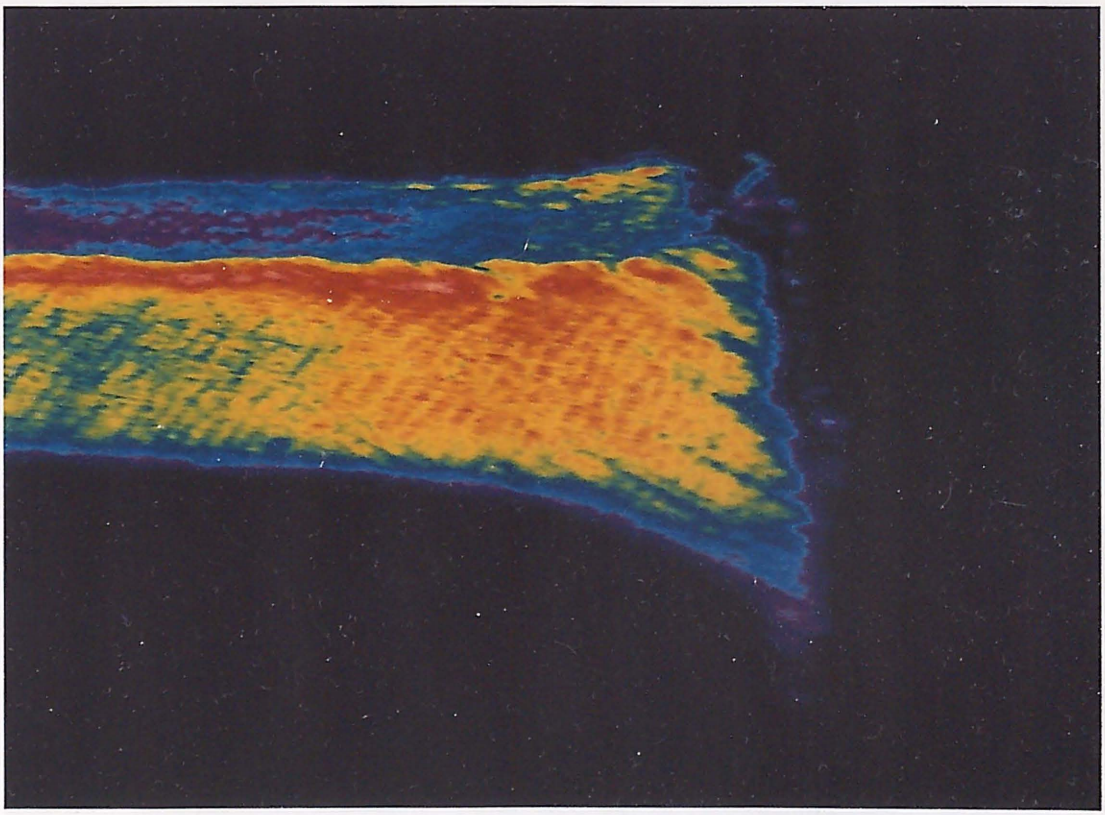


FIGURE 8. Confocal microscopy: representative image of dentin.

TABLE I

QLF: lesion area in pixels

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	317.07}	224.79	58.04	0.00	544.00
	APF pre-condensation	15	190.33	225.51	58.23	0.00	609.00
	Varnish post-condensation	15	187.73	233.32	60.24	0.00	544.00
	APF post-condensation	15	134.00	194.90	50.32	0.00	537.00
	Varnish pre-condensation	15	93.60}	153.54	39.64	0.00	523.00
Enamel	Control	15	498.87	130.00	33.57	29.00	533.00
	Varnish pre-condensation	15	527.13	47.62	12.30	363.00	574.00
	APF post-condensation	15	532.53	1.81	0.47	526.00	533.00
	Varnish post-condensation	15	533.60	2.61	0.67	532.00	543.00
	APF pre-condensation	15	534.80	7.84	2.02	530.00	563.00

* } or] indicates statistical significance ($p < 0.05$).

TABLE II

QLF: lesion area in square millimeters (mm^2)

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	0.19}]	0.12	0.03	0.00	0.30
	APF pre-condensation	15	0.10	0.13	0.03	0.00	0.30
	Varnish post-condensation	15	0.09	0.13	0.03	0.00	0.30
	APF post-condensation	15	0.07]	0.11	0.03	0.00	0.30
	Varnish pre-condensation	15	0.05}	0.09	0.02	0.00	0.30
Enamel	Control	15	0.28	0.08	0.02	0.00	0.30
	Varnish pre-condensation	15	0.29	0.03	0.01	0.20	0.30
	APF post-condensation	15	0.30	0.00	0.00	0.30	0.30
	Varnish post-condensation	15	0.30	0.00	0.00	0.30	0.30
	APF pre-condensation	15	0.30	0.00	0.00	0.30	0.30

* } or] indicates statistical significance ($p < 0.05$).

TABLE III

QLF: lesion area in square millimeters (graph)

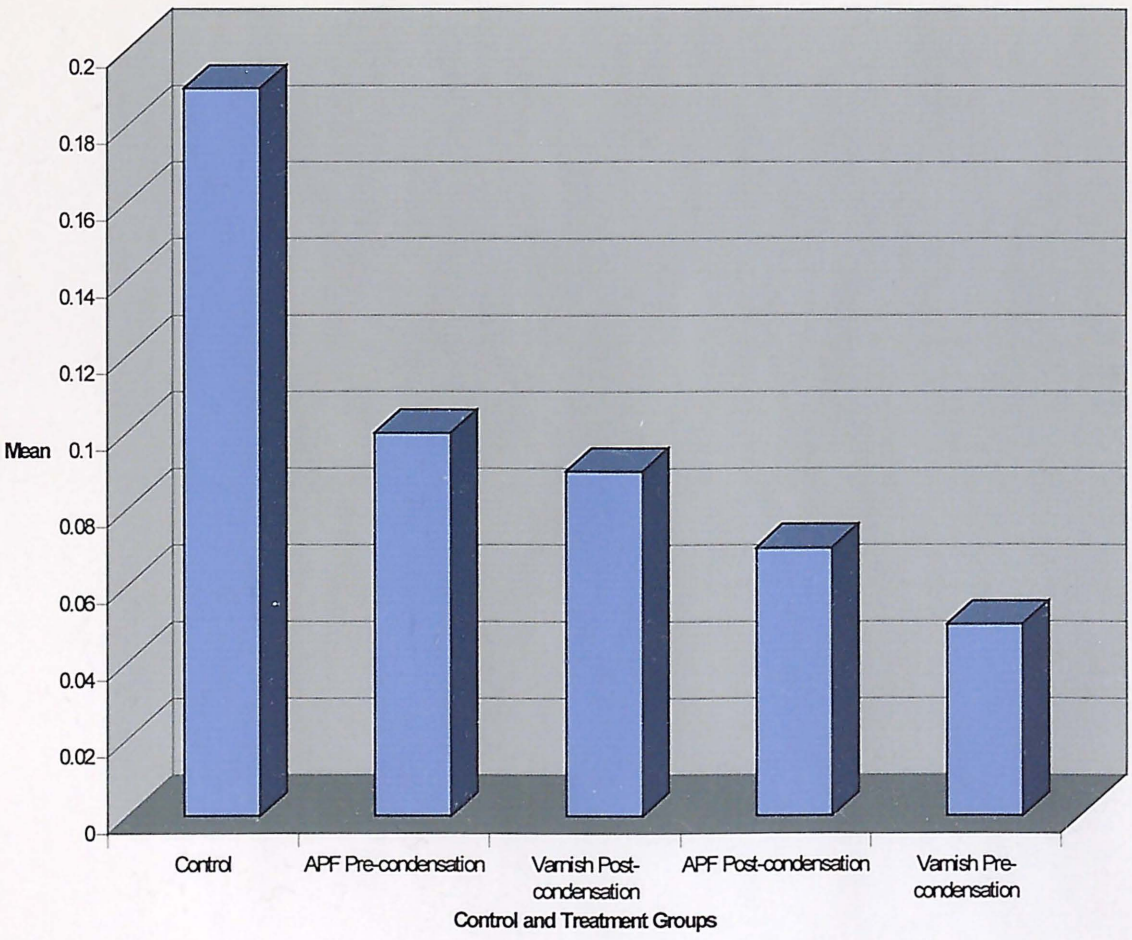


TABLE IV

QLF: maximum change in fluorescence radiance

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	-19.80}	10.83	2.80	-37.00	0.00
	Varnish post-condensation	15	-13.47	8.90	2.30	-29.00	0.00
	APF pre-condensation	15	-12.67	7.40	1.91	-25.00	0.00
	APF post-condensation	15	-12.33	9.07	2.34	-35.00	0.00
	Varnish pre-condensation	15	-9.80}	8.24	2.13	-30.00	0.00
Enamel	Varnish post-condensation	15	-50.27	11.68	3.02	-73.00	-35.00
	Control	15	-50.93	14.94	3.86	-73.00	-17.00
	Varnish pre-condensation	15	-51.53	12.82	3.31	-74.00	-35.00
	APF post-condensation	15	-52.20	9.74	2.52	-73.00	-40.00
	APF pre-condensation	15	-54.73	8.34	2.15	-69.00	-44.00

* } or] indicates statistical significance ($p < 0.05$).

TABLE V

QLF: average change in fluorescence radiance

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	-10.34}	5.38	1.39	-20.00	0.00
	Varnish post-condensation	15	-7.64	4.61	1.19	-17.40	0.00
	APF pre-condensation	15	-6.77	3.33	0.86	-11.90	0.00
	APF post-condensation	15	-6.45	3.13	0.81	-11.80	0.00
	Varnish pre-condensation	15	-5.29}	3.59	0.93	-11.10	0.00
Enamel	Control	15	-36.17	14.27	3.68	-63.00	-8.10
	APF post-condensation	15	-39.20	11.56	2.98	-61.10	-23.00
	Varnish post-condensation	15	-36.07	11.90	3.07	-59.30	-18.60
	Varnish pre-condensation	15	-37.66	14.86	3.84	-65.00	-13.10
	APF pre-condensation	15	-40.18	9.00	2.32	-55.60	-25.10

* } or] indicates statistical significance ($p < 0.05$).

TABLE VI

QLF: ^Q (ratio of fluorescence per unit area)

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	-4257.52}	3897.37	1006.30	-10880.00	0.00
	Varnish post-condensation	15	-2267.91	3270.62	844.47	-9448.20	0.00
	APF pre-condensation	15	-1791.85	2396.41	618.75	-7003.50	0.00
	APF post-condensation	15	-1250.78	2014.87	520.24	-6336.60	0.00
	Varnish pre-condensation	15	-826.84}	1527.99	394.53	-4968.50	0.00
Enamel	Control	15	-18996.03	8237.20	2126.84	-33579.00	-234.90
	APF post-condensation	15	-20882.87	6178.12	1595.18	-32566.30	-12098.00
	Varnish post-condensation	15	-19251.36	6362.96	1642.91	-31606.90	-9895.20
	Varnish pre-condensation	15	-20166.76	8402.96	2169.64	-34645.00	-4755.30
	APF pre-condensation	15	-21458.98	4716.41	1217.77	-29634.80	-14131.30

* } or] indicates statistical significance ($p < 0.05$).

TABLE VII
Polarized Light Microscopy

50 μ from the restoration

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Varnish pre-condensation	12	16808.17}	3150.18	909.38	12306.00	22419.00
	APF post-condensation	15	14815.80	3941.09	1017.59	10416.00	23742.00
	Control	14	14428.93	2922.89	781.17	8480.00	19292.00
	Varnish post-condensation	15	13591.80	3477.58	897.91	8026.00	21080.00
	APF pre-condensation	15	12401.73}	2403.39	620.55	8276.00	17194.00
Enamel	Control	13	9625.31	3435.81	952.92	4685.00	15849.00
	APF post-condensation	13	10343.77	3895.51	1080.42	4317.00	16752.00
	Varnish post-condensation	14	8993.79	2309.43	617.22	6028.00	14617.00
	Varnish pre-condensation	10	10087.30	3437.08	1086.90	3833.00	15023.00
	APF pre-condensation	11	9006.91	3911.86	1179.47	3737.00	14982.00

100 μ from the restoration

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Varnish pre-condensation	12	32746.08}	4620.40	1333.80	24512.00	40009.00
	APF post-condensation	15	27884.00}	3553.12	917.41	21729.00	35039.00
	Control	14	27621.43}	4255.94	1137.45	19027.00	34754.00
	Varnish post-condensation	15	25643.20}	4807.40	1241.27	17679.00	36588.00
	APF pre-condensation	15	25395.93}	4064.72	1049.51	16634.00	32705.00
Enamel	Control	13	18475.62	6491.34	1800.37	8506.00	30359.00
	APF post-condensation	13	20856.85	6677.96	1852.13	11005.00	31465.00
	Varnish post-condensation	14	18271.21	4035.87	1078.63	12782.00	28370.00
	Varnish pre-condensation	10	19141.80	5490.67	1736.30	9538.00	26617.00
	APF pre-condensation	11	18628.27	7604.63	2292.88	7535.00	31756.00

TABLE VIII

Polarized light microscopy:
100 μ from the restoration (graph)

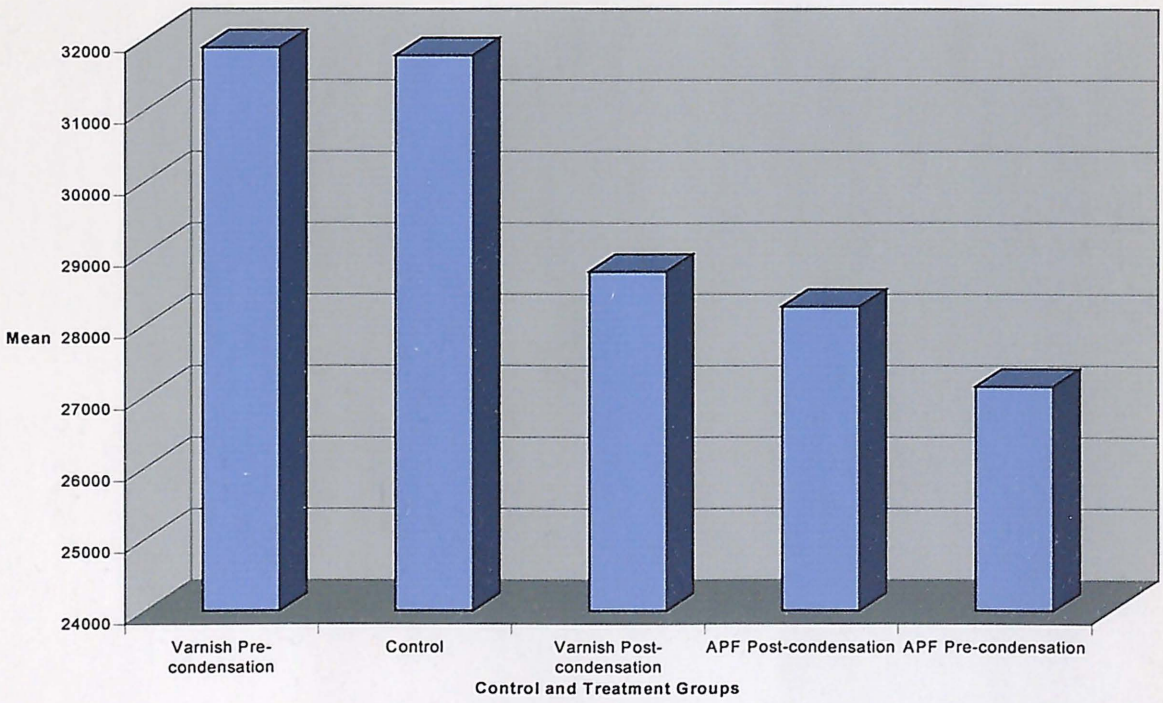


TABLE IX

Confocal microscopy area

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Varnish pre-condensation	13	31885	5531	1534	21300	42900
	Control	15	31774	6053	1563	18900	40400
	Varnish post-condensation	15	28747	2900	749	24200	33900
	APF post-condensation	15	28256	4462	1152	20400	35900
	APF pre-condensation	15	27140	3789	978	19300	32800

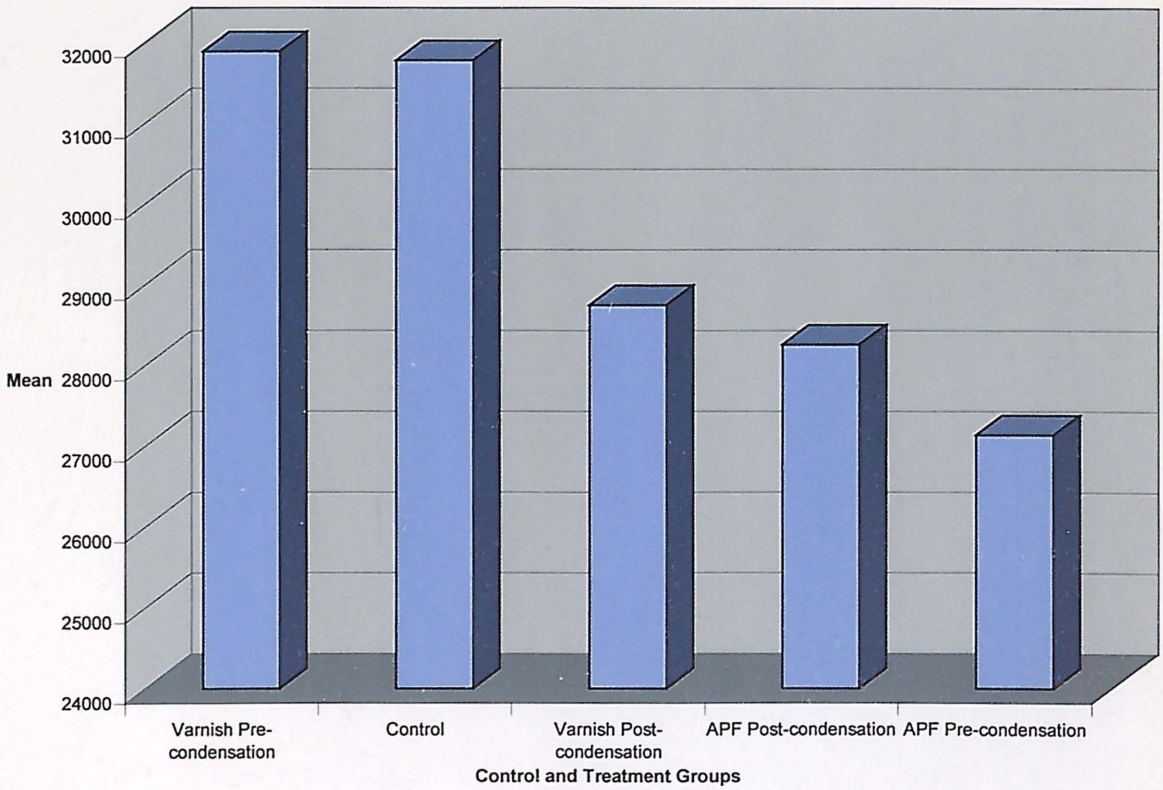
Total fluorescence

Margin	Group	N	Mean	S.D.	S.E.	Min	Max
Dentin	Control	15	3759467}	990014	255620	2350000	5760000
	Varnish pre-condensation	13	3725385	1213985	336699	1630000	5750000
	APF post-condensation	15	3267533	679431	175428	2220000	4290000
	Varnish post-condensation	15	2895733	984992	254324	1070000	4310000
	APF pre-condensation	15	2752867}	758358	195807	823000	4430000

* } or] indicates statistical significance ($p < 0.05$).

TABLE X

Confocal microscopy: area



DISCUSSION

The replacement of existing restorations accounts for 50 to 70 percent of operative dentistry today. Quantitative Light Fluorescence (QLF) has been shown to be effective at diagnosing very early tooth demineralization on smooth surfaces (less than 50 μ in depth); however, QLF has never been utilized to evaluate secondary caries in dentin. This study had several objectives. The first objective was to determine which treatment regimen would more effectively inhibit tooth demineralization: a 5.0-percent sodium fluoride varnish or a 1.23-percent APF (acidulated phosphate fluoride) foam when introduced to this evaluation system. The artificial caries system was adjusted to ensure that secondary caries would occur at restoration/tooth surface interfaces. The second objective was to determine whether placing fluoride varnish prior to amalgam condensation would reduce demineralization more effectively than an application of fluoride varnish following amalgam restoration placement. The final and main objective of this study was to validate the accuracy of QLF in diagnosing early secondary caries and then verify the results using confocal microscopy and polarized light microscopy.

This study hypothesized that the fluoride varnish would be more effective than APF foam and that both fluoride varnish and 1.23-percent APF foam would show less demineralization at the tooth restoration interface than the control. This was not the case. In examining the results and the images obtained from confocal microscopy and polarized light microscopy, it seems clear that we were too aggressive with the artificial caries system; developing secondary caries lesions were so large, comparisons could not be accurately made between groups. In retrospect it would have been beneficial to have

removed the specimens from the artificial caries system earlier and to perhaps adjusted the pH to allow for slower demineralization. The caries challenge appears to have overwhelmed the fluoride remineralization process that would have been expected from the 1.23-percent APF foam and fluoride varnish. In enamel, no statistically significant differences between the control group and the fluoride treatment groups were found, and in all the groups erosion was present. Differences between groups were seen in the dentin samples; however, they were not consistently found in all three methods of examination. The data from the current literature [14-18] clearly demonstrate that fluoride varnish and 1.23-percent APF are far superior when compared against an untreated control. This study was unable to determine whether fluoride varnish would significantly reduce secondary caries more effectively than 1.23-percent APF foam.

Although not statistically significant, the QLF analysis did show a numerical trend of the fluoride treatment regimens showing less demineralization than the control in the enamel specimens. This would correlate with what other studies have shown.[14-18] The sample preparation process that was used may explain the reason this trend was seen with QLF and not confocal microscopy or polarized light microscopy. The QLF analysis was performed prior to sectioning or placing the tooth in the casting resin. The acidic nature of the caries challenge caused the enamel to be extremely weak and friable, and when subjected to these processes, the enamel tooth structure may have been lost or destroyed before it could be evaluated by confocal microscopy and polarized light. In examining the data using confocal microscopy and polarized light microscopy, it appeared that more of the enamel tooth structure was lost or demineralized than the dentin tooth structure. This may be attributed to the differences in organic and inorganic

components in dentin and enamel. The enamel specimens' inorganic components break away when severely challenged. The collagen associated with the dentin is able to return to a hydrated form when reintroduced into an aqueous environment during microscopic analysis.

The second hypothesis of this study predicted that an application of fluoride varnish prior to amalgam condensation would result in less demineralization than an application of fluoride varnish following amalgam restoration placement. In examining the polarized light data, the dentin samples had an interesting finding both at 50 μ and 100 μ. Varnish pre-condensation showed greater demineralization than all groups including the control. It may be reasonable to assume that this occurred due to the dissolution of the varnish between the restoration and the tooth surface. The thick physical properties of fluoride varnish may cause a large gap to occur upon dissolution and allow a greater concentration of acid to create wall lesions and secondary decay when aggressively challenged.

In evaluating the placement of fluoride varnish prior to amalgam condensation, a potentially relevant clinical observation was made. Due to the adhesive nature of the varnish, carving of the amalgam restorations in the varnish pre-condensation groups was difficult and may be considered to cause an unsatisfactory result by some clinicians. It was also difficult to thin the varnish adequately before amalgam condensation. This observation raises the question of whether increased microleakage may occur once the varnish has dissolved. This appears consistent with the findings of Marchiori et al.,[51] which showed that fluoride varnish exhibited significant microleakage when the cavity margin remained in enamel.

In examining the QLF data for dentin, the control had significantly higher lesion area for pixels, maximum change in fluorescence radiance, average change in fluorescence radiance, and ^Q than varnish pre-condensation. In addition, all four treatment groups showed numerical values for the mean that exhibited less demineralization than the control.

In evaluating the polarized light data for dentin, the varnish pre-condensation showed significantly more demineralization at 50 μ than APF foam pre-condensation and had more demineralization at 100 μ than the control. There were no numerical trends with the polarized light that would indicate treatment benefits.

In examining the dentin specimens using confocal microscopy, the control showed significantly higher total fluorescence than the pre-condensation group, but not for the lesion area. There does appear to be a numerical benefit of fluoride treatment for all groups; however, varnish pre-condensation appears to have the least benefit of any treatment group.

Several points are evident from the dentin data analysis. First, the numerical trends noted are not statistically significant, but do appear consistent with the current literature. Second, if the caries challenge had been less aggressive, the results could have been clearer. We conclude that the APF foam and fluoride varnish is more effective at inhibiting secondary caries than the control. The data is not as clear concerning the placement of fluoride varnish prior to amalgam condensation. However, it does seem that fluoride varnish pre-condensation may be less effective than other treatment regimens.

The major hypothesis of this study suggested that QLF would be able to detect early incipient caries. Importantly, this study did validate the ability of QLF to detect secondary lesions. All samples were introduced to the artificial caries challenge, and all samples formed secondary caries at the restoration-tooth interface, as verified by both confocal microscopy and polarized light microscopy. QLF confirmed 100 percent of these secondary lesions; however, no sound specimens were analyzed with any of the three techniques to verify false positives. Polarized light and confocal microscopy have been very useful in helping researchers understand the caries process and incipient lesion progression. The limitation of these techniques is that they can only be used after the tooth has been removed from the oral environment. With the development of a hand-held, intraoral camera, QLF has the potential of allowing researchers and clinicians the ability to detect the lesion prior to cavitation and then monitor and treat the incipient lesions over time.

The future of QLF appears positive, and there are many areas in dentistry that may benefit from this technology. Quantitative Light Fluorescence may be especially suited for the pediatric dental setting. QLF has the ability to detect more incipient lesions than found by clinical examination, which will aid the clinician in early diagnosis.[66] This will help in identification and early treatment of those children predisposed to early childhood caries. This may be particularly true for high-risk populations that may be seen only in certain outreach centers or clinics.

This study has shown that QLF is able to confirm lesions in both enamel and dentin. With the ability to detect incipient caries in dentin, QLF may also be useful in the aging adult population for diagnosing and monitoring root caries. This may become

increasingly important as the number of adult patients at risk for root caries increases. From the results of our study, and the findings from other studies pertaining to QLF, it appears that QLF can be used in early caries diagnosis, and emphasis should now be focused on treatment of the early lesion after QLF detection.

SUMMARY AND CONCLUSIONS

This study was designed to assess *in vitro* the diagnosis and inhibition of secondary incipient caries adjacent to amalgam restorations. The primary goal was to determine if QLF could detect incipient secondary caries as accurately as the gold standards of confocal laser scanning microscopy and polarized light microscopy. A secondary goal was to examine the effect of fluoride varnish versus 1.23-percent APF foam and a control. Fluoride varnish and APF foam were applied at different points during the amalgam placement process in an effort to determine which, if any, of the treatments could reduce the formation of secondary caries most effectively.

The results of this study demonstrated that QLF detected 100 percent of the lesions seen with confocal microscopy and polarized light microscopy; however, no sound specimens were analyzed to rule out false positives. We conclude from this study that QLF can detect early caries, both in enamel and dentin, but further evaluation of the ability of this detection system to discriminate sound versus incipient lesions is warranted.

This study was unable to show a consistent, statistically significant, difference in the treatment effect of fluoride varnish versus APF foam. In general, we conclude that both fluoride treatment groups are superior to the non-treated control, and although not statistically significant, placing fluoride varnish into the cavity preparation prior to amalgam condensation may be the least effective mode of fluoride delivery.

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ABSTRACT

A COMPARISON OF A 2.26% FLUORIDE VARNISH VERSUS A 1.23% APF FOAM USING POLARIZED LIGHT MICROSCOPY, CONFOCAL MICROSCOPY AND QUANTITAVE LIGHT FLUORESCENCE

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Secondary caries and the replacement of existing restorations account for 50 to 70 percent of operative dentistry today.[1-3] Quantitative Light Fluorescence (QLF) has been shown to be effective at diagnosing very early tooth demineralization on smooth surfaces (less than 50 μ in depth); however, QLF has never been utilized to evaluate secondary caries in dentin. The objective of this study was to validate the accuracy of QLF in diagnosing early secondary caries and then verify the results using confocal microscopy and polarized light microscopy. Seventy-five mandibular molar teeth were prepared with Class V amalgam preparations on the mesial surface. A fluoridated varnish and 1.23-percent acidulated phosphate fluoride (APF) were introduced to this evaluation system[43], two agents known to effectively inhibit tooth demineralization. The artificial caries system utilized was adjusted to ensure that secondary caries would occur at restoration/tooth surface interfaces. The teeth were exposed to this artificial caries challenge for five days and following lesion formation, QLF was used to determine if

incipient demineralization could be detected. The results of the QLF analysis were then compared with the data gathered using confocal microscopy and polarized light microscopy. Our results demonstrate that QLF detected 100 percent of the lesions seen with confocal microscopy and polarized light microscopy; however, no sound specimens were analyzed with any of the three techniques. There were no consistent significant differences between the fluoridated varnish and APF ($p < 0.05$) with any of the three methods utilized. We conclude that QLF can be used in early caries diagnosis and that emphasis should now be focused on treatment of the early lesion.

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